Dietary Lactose and its Effect on the Disease Condition of Necrotic Enteritis

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ABSTRACT *Clostridium perfringens* is the etiologic agent of necrotic enteritis (NE) and is ubiquitous in nature. The incidence of NE has increased in countries and commercial companies that have stopped using antibiotic growth promoters. The mechanisms of colonization of *C. perfringens* and the factors involved in onset of NE are not fully understood. Previously, our laboratory has demonstrated that lactose could potentially reduce *Salmonella* and *C. perfringens* in ceca of poultry. In the present investigation, we hypothesized that dietary lactose would reduce the clinical signs of NE and could be used as an alternative to antibiotics. In experiment 1, day-of-hatch broilers were fed either a nonlactose control diet, a diet with 2.5% lactose, or a diet with 4.5% lactose throughout the experiment. Birds were administered *C. perfringens* (10^7 cfu/mL) daily via oral gavage for 3 consecutive days starting on d 17. When evaluating the intestinal lesions associated with NE, birds fed 2.5% lactose had significantly lower ($P < 0.05$) lesion scores (0.70 ± 0.52) compared with the control (1.55 ± 0.52) or the 4.5% lactose (1.60 ± 0.52). The data from the microbial analysis showed that the addition of lactose did not affect any bacterial populations when compared with the control birds that did not receive dietary lactose over the 21-d evaluation. The overall lesion scores in experiment 2 were significantly ($P < 0.05$) reduced in birds fed 2.5% lactose compared with the birds fed the control diet with mean lesion scores of 1.10 ± 0.73 and 1.80 ± 0.73, respectively. These experiments suggest that lactose could be used as a potential alternative to growth-promoting antibiotics to help control this costly disease.

Key words: *Clostridium perfringens*, chicken, lactose, necrotic enteritis

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INTRODUCTION

One of the key preventative measures for reducing foodborne illness in the United States is to understand the complex interactions of microorganisms that cause disease. There are many different bacteria such as *Salmonella, Escherichia coli, Clostridium perfringens,* and *Listeria monocytogenes* that contaminate poultry products. Many of these bacteria are opportunistic pathogens; however, understanding what facilitates their ability to flourish and cause disease is not completely understood.

In the poultry industry, *C. perfringens* is the etiologic agent of the disease necrotic enteritis (**NE**). Clinical signs of NE include depression, decreased appetite, decreased digestion, diarrhea, and severe necrosis of the intestinal tract (Ficken and Wages, 1997; Kaldhusdal and Løvland, 2000). The subclinical form of the disease causes a decrease in overall performance of birds and has been associated with hepatic lesions (Løvland and Kaldhusdal, 1999). In 2000, it was estimated that the subclinical form of the disease costs producers as much as 5 cents per bird due to decreased performance (Van der Sluis, 2000). Cost of this disease, including clinical and subclinical infections, was close to $2 billion dollars worldwide. *Clostridium perfringens* is prevalent in commercial poultry, with 75 to 95% of the gastrointestinal (**GI**) tract of broilers having tested positive for *C. perfringens* in previous studies (Tschirdewahn et al., 1991; Miwa et al., 1997; Craven et al., 2001a,b). Processed poultry meat has also been shown to have relatively high numbers of *C. perfringens* (Craven et al., 2001b). This enteric pathogen has been found throughout broiler production practices, with transmission to humans through the consumption of poultry products (Labbe, 1991; Craven et al., 2001a,b).

Reducing the effects of *C. perfringens* in experimental settings has been evaluated with a variety of management tools, including antibiotics, vaccines, and competitive exclusion cultures (Watkins et al., 1997; Hofacre et al., 1998; Craven et al., 1999; Brennan et al., 2003; Williams et al., 2003; Løvland et al., 2004). Currently, antibiotic growth promoters (**AGP**) are predominantly used in the commercial poultry setting and have been shown to improve the health and performance of poultry (Watkins et al., 1997; Bedford, 2000; Brennan et al., 2003). These antibiotics target gram-positive organisms, which are associated with lower

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Table 1. An evaluation of dietary lactose administration in birds experimentally infected with *Clostridium perfringens* and the development of clinical lesions associated with necrotic enteritis

	Clinical intestinal lesion scores						
Experiment 1[3]	0	1	2	3	4	Mean lesion score[1]	Mortality[2]
	(%)						
Control	0	30	50	20	0	$1.90^{A} \pm 0.76$	23/120
Lactose 1%	20	20	40	20	0	$1.55^{ABC} \pm 0.76$	18/120
Lactose 1.5%	20	40	30	10	0	$1.25^{ABCD} \pm 0.76$	13/120
Lactose 2%	30	60	10	0	0	$1.66^{AB} \pm 0.76$	14/120
Lactose 2.5%	70	30	0	0	0	$0.22^{E} \pm 0.76$	9/120*
Lactose 3%	10	70	20	0	0	$0.55^{DE} \pm 0.76$	11/120*
Lactose 3.5%	30	50	20	0	0	$0.60^{DE} \pm 0.76$	13/120
Lactose 4%	20	50	30	0	0	$0.88^{CDE} \pm 0.76$	12/120*
Lactose 4.5%	30	20	40	10	0	$1.11^{BCD} \pm 0.76$	17/120

[A–E]Mean values within the same column with no common superscripts differ significantly ($P \leq 0.05$).

[1]Lesion score is represented by the mean of the treatment subset (n = 20) with the MS error.

[2]Mortality is represented by incidence data for the experiment (n = 120).

[3]Treatment groups represented by the percentage of dietary lactose, administered in standard broiler starter feed from d 1.

*$P \leq 0.05$.

levels of performance and health. Continuing pressure to remove AGP from commercial operations could cause increased disease conditions.

Clostridium perfringens is one of the bacteria specifically targeted by AGP, and many of these products, such as avoparcin, adriamycin, bacitracin, virginiamycin, and tylosin, have already been removed from production practices in the European Union (Van Immerseel et al., 2004). Recently, one of the largest fast-food retailers in the world announced that the company plans to phase out the purchasing of animal products that have been exposed to AGP in an effort to reduce the potential effects of antibiotic resistance in many microbial populations that directly affect human medicine (CSR Wire, 2007). This food supplier has asked growers to certify that their meat products have not been treated with AGP and to maintain records of antibiotic use that would be available for company audits and reviews. In the commercial poultry industry, there has been an increase of NE in recent years in antibiotic-free flocks and some flocks that use antibiotics. Developing new intervention strategies to combat this disease condition are needed. Investigations into products that alter microbial populations in the GI tract need to be investigated.

Dietary lactose may have applications that may be beneficial in reducing the effects of this disease. Lactose is a disaccharide that naturally occurs in mammalian milk. Lactose or milk sugar can be broken down into its 2 smaller components of galactose and glucose by an enzyme called lactase. In 2002, approximately 563 million pounds of lactose were produced in the United States, of which 118 million pounds were exported. The increase in production of lactose has led to developing or investigating new uses for this product. *Clostridium perfringens* can ferment lactose, and it has been shown that lactose can significantly reduce colonization in the ceca of chickens (Takeda et al., 1995). Lactose has also been shown to have beneficial effects on the GI microflora. An investigation in day-of-hatch chicks found that dietary lactose decreased *Lactobacillus, Clostridium,* and *Proteus* species and increased bifidobacteria in the ceca (Morishita et al., 1982; Van der Wielen et al., 2002). Our laboratory is currently interested in the investigations of lactose and its effect on the microbial populations of the GI tract during NE. The objective of the present investigation was to evaluate the microbial ecology and clinical signs associated with NE in the GI system of birds being fed dietary lactose.

MATERIALS AND METHODS

Experimental Birds

Ross × Ross straight-run broiler chicks were obtained from a local commercial hatchery on the day of hatch and were placed on clean pine shaving litter. Birds were reared in 2.4 × 1.2 m pens, allowing 0.12 m^2 of pen space per bird. Chicks were provided with water and a 55% wheat-corn-based broiler starter diet ad libitum. High concentrations of wheat in the diet have been shown to exacerbate the outbreak of NE (Johnson and Pinedo, 1971; Truscott and Al-Sheikhly, 1977; Branton et al., 1987; Riddell and Kong, 1992). The diet met or exceeded NRC guidelines for broiler chicks (NRC, 1994).

Experimental Design

In experiment 1, birds were randomly assigned to one of the following groups: negative control (normal broiler starter diet) or 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, or 4.5% dietary lactose. Birds were fed the control or dietary lactose diets from the day of hatch until termination of the experiment. In experiment 2, birds were randomly assigned to one of the following groups and fed one of the following diets: negative control (normal broiler starter diet) or 2.5% dietary lactose.

In experiment 1, we evaluated several parameters on 7, 14, and 21 d of age. We evaluated the effects of dietary lactose on mortality, microbial populations (n = 20), intesti-

Table 2. An evaluation of select microbial populations in birds fed dietary lactose in association with necrotic enteritis

	Enterococcus			Lactobacilli			*Clostridium*			*Escherichia coli*		
Experiment 1[1]	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
	(mean $\log_{10}$)											
Control	6.22 ± 0.32[2]	5.93 ± 0.70	5.70 ± 0.47	7.62 ± 0.25	7.67 ± 0.48	7.25 ± 0.61	1.21 ± 0.65	1.10 ± 0.64	5.54 ± 0.58	5.01 ± 0.81	4.67 ± 0.53	5.98 ± 0.56
Lactose 2.5%	4.92 ± 0.32	5.53 ± 0.70	4.94 ± 0.47	7.56 ± 0.25	7.28 ± 0.48	7.91 ± 0.61	1.31 ± 0.65	1.05 ± 0.64	5.74 ± 0.58	5.46 ± 0.81	5.80 ± 0.53	5.19 ± 0.56
Lactose 4.5%	5.70 ± 0.32	4.94 ± 0.70	5.95 ± 0.47	7.25 ± 0.25	7.91 ± 0.48	6.93 ± 0.61	1.00 ± 0.65	1.23 ± 0.64	6.25 ± 0.58	4.50 ± 0.81	3.86 ± 0.53	6.09 ± 0.56

[1]Treatment groups represented by the percentage of dietary lactose, administered in standard broiler starter feed from d 1.
[2]$\log_{10}$ values are represented by the mean of the treatment subset (n = 10) ± the SE.

nal pH (n = 5), and the development of clinical lesions (n = 20, d 21). In experiment 2, two replicate studies were run to evaluate the effects of dietary lactose on the development of clinical lesions (n = 35, d 21) and mortality. The data from these replicate studies were pooled and reported.

Immunosuppression Vaccine Administration

As previously described, a commercial bursal disease vaccine was used as an immunosuppressant in the present investigations (McReynolds et al., 2004). All experimental birds were administered the vaccine on d 14 at a level 10× the recommended dose of the manufacturer via ocular route to immunocompromise the chicks. Challenge doses, at these concentrations, were chosen based on previous research (data not shown) and have been known to show signs of the disease state.

C. perfringens Administration

Four field isolates of *C. perfringens* (type A) from different geographical locations (1 isolate from Texas and Virginia and 2 isolates from Georgia) were cultured separately then combined and provided to the appropriate treatment groups (McReynolds et al., 2004). For challenge, the isolates were grown in thioglycollate medium for 12 h, and the chicks were challenged via oral gavage (3 mL) with 10^7 cfu of *C. perfringens*/mL. Birds were administered *C. perfringens*, beginning on d 17, twice daily for 3 d. Challenge doses, at these concentrations, were chosen based on previous research (data not shown) and have been known to show signs of the disease state with intestinal lesions.

Bacterial Culture

To quantitatively measure populations of *C. perfringens, E. coli, Enterococcus*, and lactobacilli, a section of the small intestine about 6 in. (15.24 cm) in length, just cranial to Meckel's diverticulum, was removed. The sample was placed in 10 mL of anaerobic thioglycollate, stomached for 30 s, and 0.5 mL of gut contents was removed and placed into 4.5 mL of thioglycollate media (*C. perfringens* only) or neutral PBS (all other bacteria). Ten-fold serial dilutions were performed and plated on Shahidi Ferguson Perfringens, MacConkey, M-*Enterococcus,* and lactobacilli de-Man, Rogosa, and Sharpe agar for *C. perfringens, E.coli, Enterococcus,* and lactobacilli, respectively, and incubated (24 h at 37°C). All the *C. perfringens* and lactobacilli culture work was performed in an anaerobic hood. Colonies exhibiting typical colony morphology for each species were counted and recorded.

NE Lesion Scores

To evaluate gross lesions associated with NE, the jejunum and ileum of the small intestine were examined. Lesion scores were recorded using the following criteria (Prescott et al., 1978): 0 = no gross lesions, normal intestinal

Table 3. An evaluation of intestinal pH in the duodenum and jejunum in birds fed dietary lactose in association with necrotic enteritis

	Upper intestinal pH[1]					
	7 d		14 d		21 d	
Experiment 1[2]	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum
Control	6.01 ± 0.07	5.46 ± 0.17	5.96 ± 0.06	5.82 ± 0.08	6.03 ± 0.14	6.02 ± 0.08
Lactose 1%	5.92 ± 0.07	5.78 ± 0.31	5.98 ± 0.04	5.49 ± 0.02	6.07 ± 0.13	6.02 ± 0.06
Lactose 1.5%	5.96 ± 0.10	5.41 ± 0.26	5.95 ± 0.05	5.64 ± 0.14	5.78 ± 0.16	6.06 ± 0.02
Lactose 2%	6.00 ± 0.05	5.65 ± 0.08	6.01 ± 0.05	5.63 ± 0.13	5.57 ± 0.17	5.93 ± 0.11
Lactose 2.5%	5.90 ± 0.02	5.79 ± 0.15	5.92 ± 0.07	5.83 ± 0.22	5.25 ± 0.35	6.02 ± 0.06
Lactose 3%	6.00 ± 0.04	6.23 ± 0.21	5.85 ± 0.06	5.65 ± 0.27	5.60 ± 0.10	5.99 ± 0.08
Lactose 3.5%	5.93 ± 0.06	6.19 ± 0.31	5.88 ± 0.03	5.88 ± 0.16	5.72 ± 0.07	6.05 ± 0.03
Lactose 4%	5.92 ± 0.05	6.18 ± 0.15	5.84 ± 0.07	5.77 ± 0.17	5.48 ± 0.10	5.97 ± 0.08
Lactose 4.5%	6.00 ± 0.05	5.96 ± 0.16	5.94 ± 0.02	5.64 ± 0.12	5.52 ± 0.21	6.08 ± 0.01

[1]Intestinal pH is represented by the mean of the treatment subset (n = 5) with the MS error.

[2]Treatment groups represented by the percentage of dietary lactose, administered in standard broiler starter feed from d 1.

appearance; 1 = thin-walled or friable, gray appearance; 2 = thin-walled, focal necrosis, gray appearance, small amounts of gas production; 3 = thin-walled, sizable patches of necrosis, gas-filled intestine, small flecks of blood; and 4 = severe extensive necrosis, marked hemorrhage, much gas in intestine.

Statistical Analysis

Mortality among all treatment groups was compared using the χ^2 test of independence ($P \leq 0.05$). All mortality data were based on the total number of birds per treatment group for individual experiments; all the other measured parameters were subsets of treatment groups for individual experiments. Bacterial counts ($\log_{10}$ units) were analyzed with the PROC MIXED procedure in SAS and adjusted for multiple comparisons using the Tukey option. To evaluate the lesion scores in the present investigation, the row mean scores were compared using the Cochran-Mantel-Haenszel test and PROC FREQ. The Cochran-Mantel-Haenszel test showed significant differences ($P \leq 0.05$), and the data were further analyzed using a nonparametric ANOVA (Kruskal-Wallis) by ranking the scores, applying the mean to ties, and running a PROC GLM on the ranks, allowing the treatment groups to be compared by the mean ranks (SAS Institute, 1996).

RESULTS AND DISCUSSION

In experiment 1, we evaluated many different levels of dietary lactose to establish a dose response curve for efficacy in NE intestinal lesion development. We chose to evaluate levels less than 5%, because high levels of lactose (7 to 10%) have been shown to increase the incidence of diarrhea, which results in wet litter and increased caking of the litter (Waldroup et al., 1992). None of the experimental groups in this study had any noticeable change in the viscosity of the feces. The data from experiment 1 showed that dietary lactose fed at 2.5% was most efficacious in reducing the clinical signs of NE. When evaluating the percentage of clinical intestinal lesion scores (Table 1), it is important to note the shift in lesion scores compared between the 2.5% lactose and controls. All the birds fed the control diet (100%) had signs of clinical intestinal lesion scores when compared with the birds fed the 2.5% lactose diet (70%), which had no clinical lesion scores at all. These data show that dietary lactose can significantly alter the severity of intestinal lesion scores in birds with NE. The overall lesion scores in experiment 1 were significantly ($P > 0.05$, n = 20) reduced in birds fed the 2.5% lactose diet when compared with the control birds or birds fed 4.5% lactose with mean lesion scores of 0.22 ± 0.76, 1.90 ± 0.76, and 1.11 ± 0.76, respectively (Table 1). Mortality was also

Table 4. An evaluation of dietary lactose administration in birds experimentally infected with *Clostridium perfringens* and the development of clinical lesions associated with necrotic enteritis

	Clinical intestinal lesion scores						
Experiment 2[1]	0	1	2	3	4	Mean lesion score[2]	Mortality[3]
	(%)						
Control	3	30	54	10	3	1.80^{A} ± 0.732	35/150
Lactose 2.5%	24	49	23	1	3	1.10^{B} ± 0.73	14/150*

[A,B]Mean values within the same column with no common superscripts differ significantly ($P \leq 0.05$).

[1]Treatment groups represented by the percentage of dietary lactose, administered in standard broiler starter feed from d 1.

[2]Lesion score is represented by the mean of the treatment subset (n = 70) with the MS error.

[3]Mortality is represented by incidence data for the experiment (n = 150).

*$P \leq 0.05$.

significantly reduced ($P > 0.05$) by the addition of 2.5% dietary lactose (9/120) when compared with the control (23/120). Reducing the severity of these intestinal lesions does have an effect on the physiological structure of the intestine. Maintaining intestinal integrity and functionality will improve the ability of birds to overcome the disease. If a commercial broiler house had a mild subclinical form of the disease, lactose could help in lowering the severity of the disease state. This could potentially save the poultry industry an enormous amount of money that is currently lost to this disease.

Apajalahti and Bedford (2000) showed the relative abundance of the following bacterial populations of *E. coli*, *Enterococcus*, *Proteus*, and *C. perfringens* in birds that had NE. We evaluated these populations of bacteria to see whether we could significantly alter one of them by feeding dietary lactose. For the purpose of this experiment, we evaluated lactobacilli instead of *Proteus.* The data from the microbial analysis showed that the addition of lactose did not affect any bacterial populations when compared with the control birds that did not receive dietary lactose (Table 2) over the 21-d evaluation. Other investigations observing the effect of lactose on the GI microflora have found that lactose decreased *Lactobacillus*, *Clostridium*, and *Proteus* species and increased bifidobacteria in the ceca (Morishita et al., 1982; Van der Wielen et al., 2002). The results of these studies concluded that *Lactobacillus* species increased lactate concentrations but did not affect acetate and propionate in the ceca. Some of these populations of bacteria have been previously reported to increase during NE outbreaks; therefore, we evaluated their interactions in the GI tract during the disease state. Although none of the microbial populations dramatically changed over the course of this experiment, the ecological diversity of the GI tract during the disease state continues to be an area of interest.

Previous research in our laboratory showed that feeding dietary lactose would promote lactobacilli species to develop throughout the intestinal tract. We hypothesized that the increase in lactobacilli would promote the production of lactate, and the pH of the intestinal tract would decrease, negatively affecting certain bacteria, including *C. perfringens* (Corrier et al., 1990). The results from experiment 1 demonstrated that the pH in the duodenal loop and in the ileum was not significantly affected at 7, 14, or 21 d (Table 3). Our data does not follow the same trends seen with other pathogens. Tellez et al. (1993) found that feeding 10% lactose in the diet resulted in a significant increase in volatile fatty acids, which resulted in a significant decrease in pH and *Salmonella* Enteritidis invasion of Leghorn organs. Similar results have been reported when lactose was provided in the drinking water (2.5%) or in the feed (5 or 10%), showing significant increases in bacteriostatic acetic and propionic acids and decreases in cecal pH (Corrier et al., 1990). Although the data in this study did not show a pH decrease, it is important to remember that NE is a very complex disease. Further investigations using molecular techniques will help us understand the complexity between the bacterial and physiological interactions in the GI tract.

In experiment 2, when evaluating the percentage of clinical intestinal lesion scores (Table 4) a noticeable shift in lesion scores occurred. There was a major shift in the percentage of birds that were not clinically infected (lesion score of 0) in birds fed 2.5% dietary lactose (24%) compared with birds fed the control diet (3%). An inverse relationship was also observed in lesion score ranking 1 and 2 between the control group and the birds fed 2.5% dietary lactose. This inverse relationship in lesion scores shows that feeding 2.5% dietary lactose will lower these clinical lesions, which will improve the recovery time of the bird from the disease. The overall mean lesion scores in experiment 2 were significantly ($P > 0.05$) reduced in birds fed 2.5% lactose compared with the birds fed the control diet with mean lesion scores of 1.10 ± 0.73 and 1.80 ± 0.73, respectively (Table 4). Mortality was also significantly altered by the addition of 2.5% lactose (14/150) compared with controls (35/150). When evaluating the data from these experiments, clearly the addition of dietary lactose at the 2.5% dose greatly reduced the clinical signs of this disease.

The mechanism of action of dietary lactose on NE has been debated for some time. Early studies have suggested that carbohydrates inhibit the adherence of bacteria to epithelial cells (Swanson, 1973; Ofek et al., 1975; Jones and Freter, 1976; Oyofo et al., 1989b). Takeda et al. (1995) have shown that birds fed dietary lactose at 2 or 10% had significant reductions of *C. perfringens* in the ceca; this effect was presumably from the fermentation of lactose (Morishita et al., 1982; Corrier et al., 1990). This effect could happen in the GI tract, leading to the reduced lesion scores that were seen in the present investigation. In 1989, Oyofo et al. (1989a) found that mannose (2.5%) or lactose (2.5%) provided in the water significantly reduced the incidence of cecal *Salmonella* in chicks by 50% compared with the controls. The results of this study suggest that specific carbohydrates can be used to control *Salmonella* in day-of-hatch chicks. Another study by Tellez et al. (1993) showed that broilers fed 10% lactose had marked reductions in the lamina propria thickness of the ceca. This may be due to the osmotic effect of the lactose in the GI tract or an increase in gas production and distension of the cecal wall. The current literature suggests that lactose does affect the colonization of some pathogens in the GI tract; understanding the ecological parameters of these populations might provide insight into new technologies.

There are a myriad of physiological parameters that need to be evaluated to completely understand the interactions of dietary lactose and NE. Dietary lactose has been shown to significantly increase many key attributes in the poultry industry, from increasing eggshell quality to the prevention of pathogenic bacteria causing disease. Furthermore, it has been shown to significantly reduce the effects of *C. perfringens* in the present investigations. Dietary lactose provides the poultry industry with an alternative that has the potential to promote better animal health and decrease monetary losses due to NE.

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